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ORIGINAL PAPER

Morpho-functional changes of fat body in bacteria fed *Drosophila melanogaster* strains

Antonella Franchini · Mauro Mandrioli · Claudio Franceschi · Enzo Ottaviani

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Abstract We have examined the addition of *Escherichia coli* to the diet at day 0 of adult life of females from two Oregon R *Drosophila melanogaster* strains, selected for different longevities: a short-life with an average adult life span of 10 days and a long-life standard R strain with an average adult life span of 50 days. The addition of bacteria to the diet significantly prolonged the fly longevity in both strains and affected the structure and histochemical reactivity of the fat body. The increased survival was characterized by great amount of glycogen accumulated in fat body cells from both strains. In aged control animals, fed with standard diet, lipid droplets were seen to be stored in fat body of short-lived, but not long-lived, flies. On the whole, our data indicate that exogenous bacteria are able to extend the survival of *Drosophila* females, and suggest that such a beneficial effect can be mediated, at least in part, by the fat body cells that likely play a role in modulating the accumulation and mobilization of reserve stores to ensure lifelong energy homeostasis.

Keywords *Escherichia coli* · *Drosophila melanogaster* · Longevity · Fat body · Morphology · Histochemistry

A. Franchini · M. Mandrioli · E. Ottaviani (✉)
Department of Biology, University of Modena and Reggio Emilia, Via Campi 213/D, 41125 Modena, Italy
e-mail: enzo.ottaviani@unimore.it

C. Franceschi
Department of Experimental Pathology and CIG, Interdipartimental Center L. Galvani, University of Bologna, Bologna, Italy

Introduction

Studies performed in worms, flies and humans suggest an important role of gut microbiota in aging and longevity (Ottaviani et al. 2011). In particular, bacteria are found to be associated with the gut of several insect species (Bignell 1984; Campbell and Bernays 1990; Dillon and Dillon 2004; Dillon et al. 2010) and they may be permanently or transiently present during some life stages (De Vries et al. 2008). The gut microbial diversity includes beneficial symbionts, non-symbionts, food-borne microbes and life threatening pathogens, affects gut structure and nature of gut lining and is related to environment exposure (diet, season, pH, redox conditions) (Breznak and Panratz 1977; Bignell 1984; Lee 1980, 1985; Campbell and Bernays 1990; Dillon and Dillon 2004). The gut epithelia actively takes part in the microbe homeostasis by shaping the commensal community while contrasting pathobionts, however the molecular regulatory mechanisms involved in maintenance of the well balanced interactions are not completely understood (Lee 2008; Charroux and Royet 2010; Ryu et al. 2010). Several functions have been described for this complex and dynamic microbial ecosystem that is not only an important component of innate system, but it can modify the function of the digestive tract and the nature of nutrients available to the host (Brauman et al. 2001; Xu and Gordon 2003; Dillon and Dillon 2004; Ha et al. 2005; Rajagopal 2009; Charroux and Royet 2010). A role in supporting insect growth and development has also been reported and studies performed in different insect species revealed that exogenous bacteria can increase longevity (Brummel et al. 2004; Behar et al. 2008).

A pivotal organ involved in the control of aging and lifespan is the adipose tissue with the insulin/insulin like growth factor signalling (IIS) playing a crucial role

(Klöting and Blüher 2005). Mutations that reduce the activity of IIS extend longevity in *Drosophila* and may increase the storage of nutrients (Tatar et al. 2001; Longo and Finch 2003; Katic and Kahn 2005; Fontana et al. 2010). A reduction in mass of the fat body, a fly organ that acts as nutrient sensor, was also observed (Colombani et al. 2003; Klöting and Blüher 2005; Blüher 2008). As a human counterpart, specific polymorphisms of insulin-like growth factor 1 receptor are correlated with longevity in centenarians who are characterized by absence of insulin resistance and low body mass index (Paolisso et al. 2001; Bonafè et al. 2003).

In the present paper we studied the effect of exogenous bacteria, *Escherichia coli*, on the lifespan of two strains (short- and long-life) of *Drosophila melanogaster* in relation to the possible morphological and histochemical modifications in fat body.

Materials and methods

Flies and food preparation

Two strains of Oregon R *D. melanogaster,* selected for different longevities according to high life span variations (Lints et al. 1989), were used in this investigation: a short-life strain (Bloomington *Drosophila* Stock Center FBst0006971) with an average adult life span of 10 days and a long-life standard lived R strain with an average adult life span of 50 days. Flies were grown on "Formula4-24® Blue *Drosophila*" medium (Carolina Biological Supply Company, Burlington, NC 27215, USA) at 23 ± 1°C. Fly stocks have been synchronized starting the experiments by collecting eggs for 4 h. Adults hatched from pupae were used.

Lifespan measurements

In order to evaluate the effect of exogenous live bacteria, *Escherichia coli* cultures have been grown in LB medium, resuspended in PBS in order to have a 10^9 cells/ml. Fifties µl of bacterial solution have been added to the medium of the two fly strains, whereas in the control fly culture 50 µl of PBS have been added. Each experiment has been performed using 30 adult *Drosophila* females per vial immediately after their emergence from pupae (day 0 of adult life). After bacterial induction, alive flies have been counted daily up to the death of all the flies in each vial. The recorded number has been evaluated as percentage of alive flies and plotted with Excel 2007 software in order to evaluate the lifespan in controls and in the flies challenged with *E. coli*. The experiment has been repeated ten times. Statistical significance has been evaluated using the Log-rank test (Bland and Altman 2004).

Histology and histochemistry

Drosophila females fed by *E. coli* and control females fed by standard diet were collected from both strains, on the basis of lifespan measurement data (Fig. 1A, B), at day 4, and 9 of adult life for the short-life strain, and at day 20, 43, 45 and 48 of adult life for long-life strain. The flies were immediately fixed in Gendre's mixture (for glycogen detection) and Ca-formol (for lipids) and embedded in agar/paraffin, as previously described (Franchini et al. 2003). On 7 µm transversal or longitudinal paraffin serial sections, histological stain (hematoxylin-eosin) and specific histochemical reactions to study the presence of glycogen (sections stained with PAS/hematoxylin reaction were compared to control sections pre-treated with amylase solution), proteins (Bromophenol blue) and lipids (Sudan black B) were performed (Bancroft and Gamble 2002). Sudan black B reaction was also performed on frozen sections.

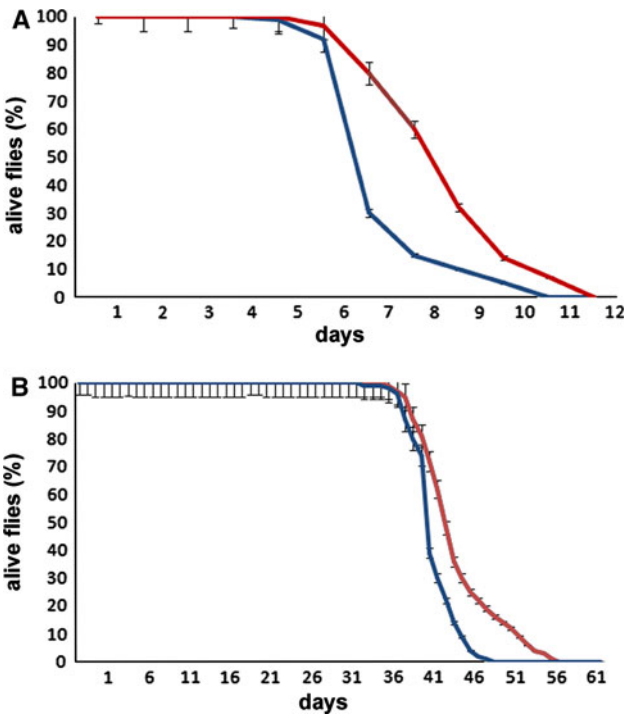

Fig. 1 Analysis of the longevity of the short-life (**A**) and long-life (**B**) fly strains. Comparison of the percentages of alive *Drosophila* females in the control flies (*black lines*) and after bacterial challenges (*gray lines*) shows that exposure to exogenous bacteria enhance the survival in both strains. Statistical comparison has been performed using the Logrank test in both short-life strain (KM = 17.24, $P > 0.001$) and long-life strain (KM = 20.37, $P > 0.001$)

Results

E. coli enhances the fly longevity

The addition of *E. coli* to the diet of female *Drosophila* significantly enhanced the longevity of both *Drosophila* strains (Fig. 1A, B). In the short-life flies, the extension of lifespan started at day 5 and became particularly evident at day 7 and 9, being the number of survived flies grown in presence of bacteria four and three times higher than controls, respectively. The flies from control flies were all dead at day 11, when 5% of flies fed *E. coli* was still alive (Fig. 1A). In the long-life strain, an extension of lifespan was also observed. Differences in survival were first detected at day 41 and at day 45 and 48 the percentage of survived bacteria fed flies was three and five times higher than controls, respectively. All control flies were dead between the 49th and 51st day when about 20–25% of flies grown in presence of bacteria were still alive (Fig. 1B).

The addition *E. coli* to the diet affected the morphological and histochemical patterns of the fly fat body that, in both Oregon R stains, showed the typical structural organization described in several insect species (Roma et al. 2010). The organ is a loose tissue formed by both layers of cells close to the integument and different sized lobes suspended in hemocel surrounding the internal organs present in thorax and abdomen cavities, such as gut and gonads. In different aged flies, it appeared highly dynamic, heterogeneous in structure and constituted by polygonal cells, the most abundant cell type, containing different amounts of stored materials.

At day 4, in most (about 85%) of control females from short-life strain, the fat body was well developed with large cells filled with glycogen whose PAS positivity (Fig. 2A, B) was abolished by amylase digestion (Fig. 2C, D), and containing few empty unstained vacuola of different size (Fig. 2B). Within such PAS positive cells no lipid droplets were detected, but small scattered cytoplasmic granula and perinuclear areas were stained with the Bromophenol blue reaction specific for proteins (Fig. 2E, F). The oenocytes, easily distinguishable for their staining properties, *i.e.,* a basophilic cytoplasm intensely reactive to Bromophenol blue (Fig. 2E) and Sudan black B (Fig. 2G), were found grouped under the integument and distributed among the perivisceral lobes. Some control flies (about 10%) exhibited fat bodies with regional differences distinguished by morphological stains and histochemical reactions. Cell layers arranged in the parietal areas containing vacuolated PAS positive cells and a network of loose perivisceral lobes containing cells poor in glycogen and rich in lipid droplets were observed (Fig. 3A, B). At day 4, when no difference in survival was observed, the structure and histochemical reactivity of fat body from samples fed by *E. coli* were similar to controls, except for the presence of lobes of cells containing lipid droplets that were not detected (Fig. 3C–F).

At day 9, in the majority of control survived flies the fat body showed a looser structure reduced in its perivisceral lobes with vacuolated cells mainly storing lipid droplets (Fig. 4A). Glycogen storing was sometimes seen in thin parietal areas (Fig. 4B). In contrast, fat bodies from most flies grown in the presence of bacteria contained well developed islets of cells, full of glycogen, associated to segmentally arranged oenocytes (Fig. 4C–E). In some flies, perivisceral cell aggregates were seen to contain lipid droplets (Fig. 4F).

With regards the long-life strain, at day 20 and 43 no significative differences were observed in the structure and histochemical reacticity of fat body from flies fed with different diets and they were similar to those of short lived flies. When the longevity was significantly extended, this strain was characterized by an higher percentage of control flies containing decreased fat body glycogen stores than bacteria fed flies (Fig. 5A, B). The fat body cells were PAS-negative in 50% of control survived flies against a 25% of bacteria fed flies at day 45, and in 85% of controls against a 55% at day 48. Moreover, in contrast to the short lived strain, no lipid droplets were seen to accumulate in empty vacuolated cells in the course of animal aging (Fig. 5A, B).

Discussion

The major finding of the present paper is that the addition of exogenous *E. coli* to the diet of two different longevity strains of *Drosophila* females at day 0 of adult life increases their survival. The effect of such a dietary intervention became marked at day 7 and 9 in short-life strain when the number of *E. coli* supplemented flies was four and three times higher than controls fed by the standard diet and at day 45 and 48 for long-life strain with percentages of survived bacteria fed flies three and five times higher than controls, respectively.

The digestive tract of many insect species harbours several bacteria that perform different functions beneficial to their host, and the interactions between dominant and minor microbial communities within the gut may affect host longevity. In *Ceratitis capitata* fed with Enterobacteriaceae the lifespan was extended and the bacteria were found to contribute to medfly metabolism, development, copulatory success and to contrast deleterious bacteria (Behar et al. 2008). In *Drosophila,* non-virulent strains of *Wolbachia* can extend lifespan (Fry and Rand 2002), and experiments performed by using axenic cultures and antibiotic treatment revealed that exposure to bacteria during

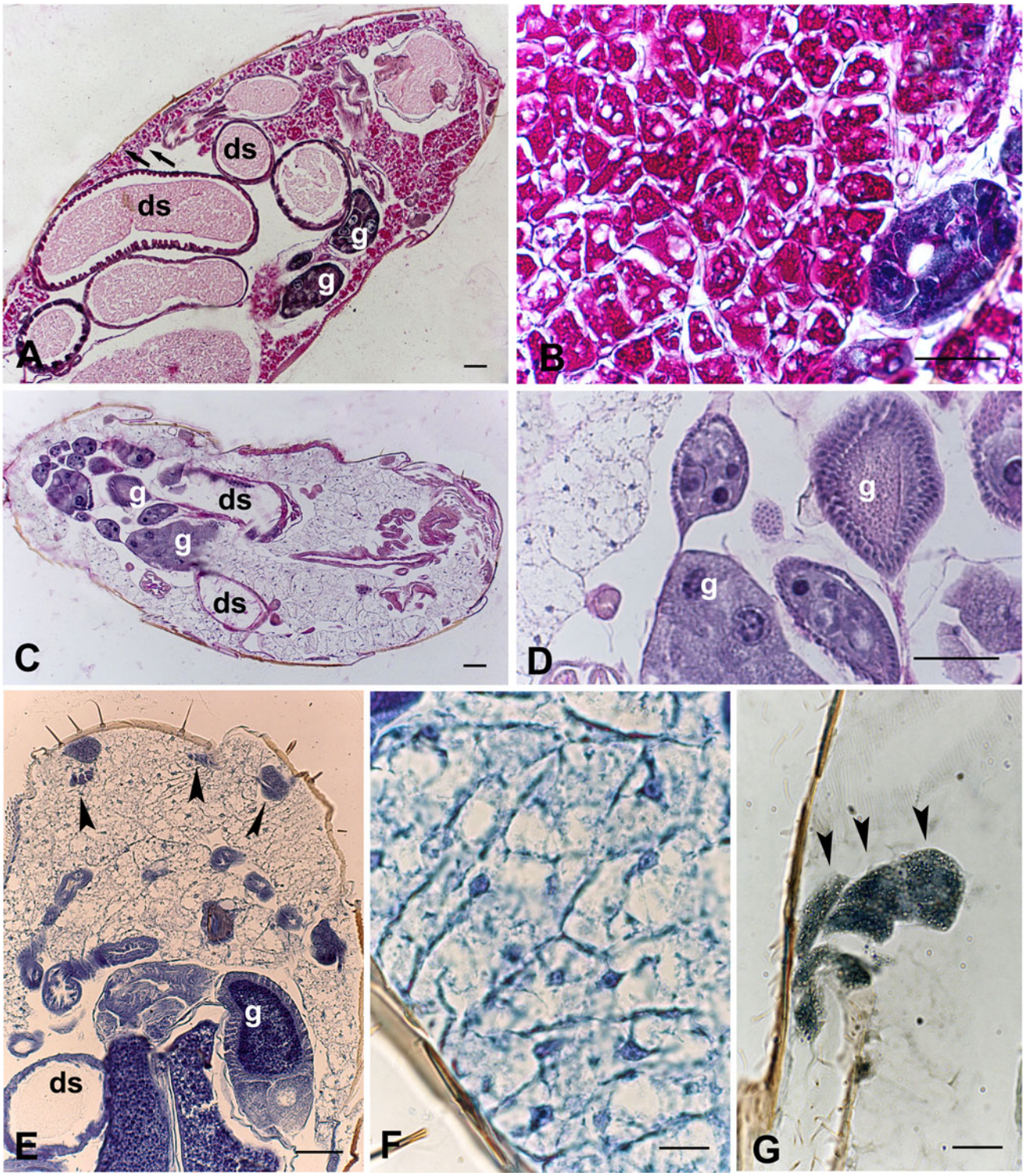

Fig. 2 Histology of the fat body from 4 day old short-life *Drosophila* females fed by standard diet (controls). Longitudinal sections stained with PAS/hematoxylin (**A**, **B**), PAS/hematoxylin after amylase digestion pre-treatment (**C**, **D**), Bromophenol blue (**E**, **F**) and Sudan black B reactions (**G**). **A**–**G** Fat bodies are characterized by cell layers close to the integument (*arrows*) and perivisceral lobes surrounding the internal organs, such as digestive system (ds) and gonads (*g*). The cells store mainly glycogen and few empty unstained vacuola. The oenocytes are indicated by *arrowheads*. *Bars* 50 μm (**A**–**E**), 10 μm (**F**, **G**)

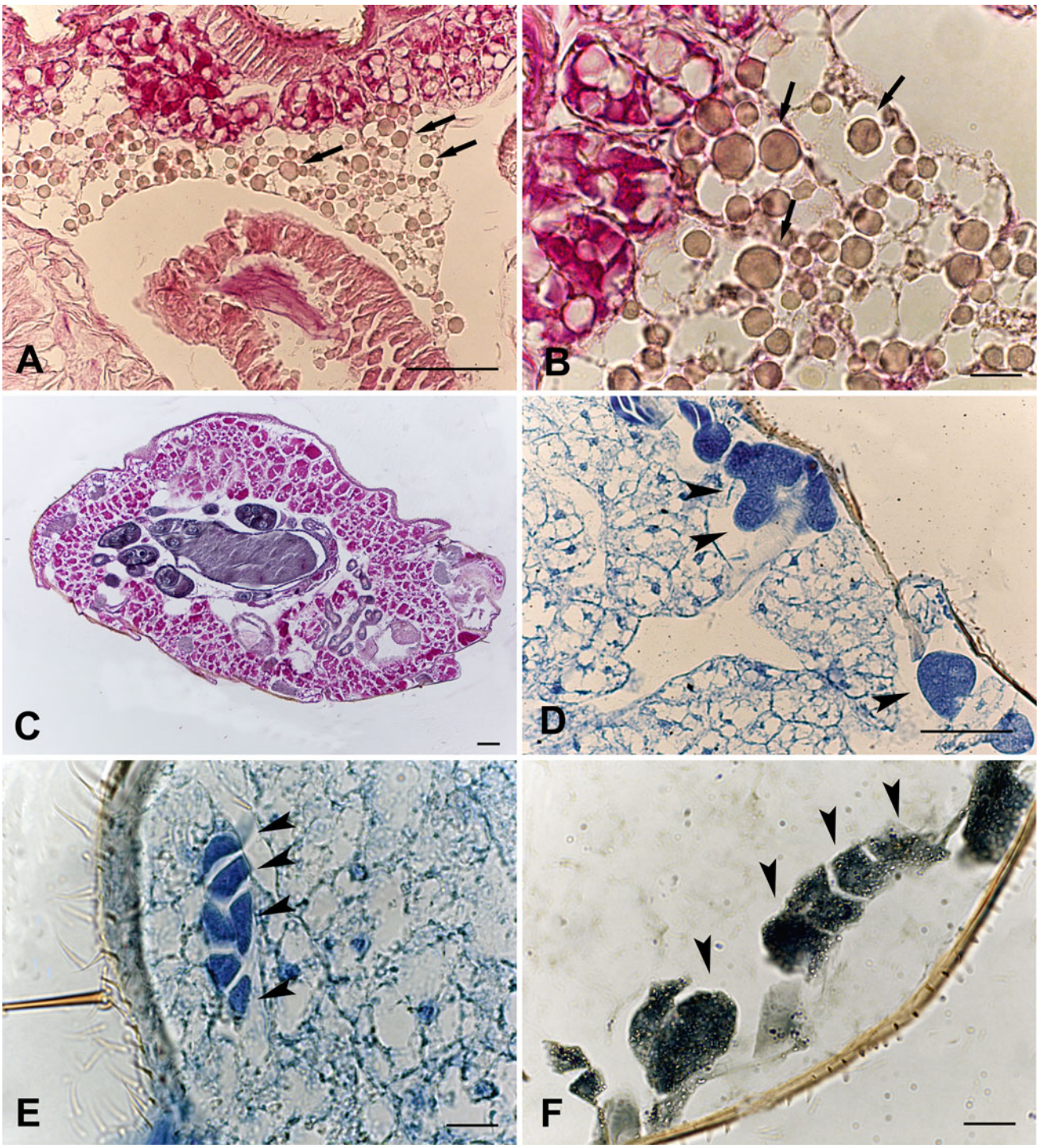

Fig. 3 Histology of the fat body from 4 day old short-life *Drosophila* females. Longitudinal sections stained with PAS/hematoxylin (**A**–**C**), Bromophenol blue (**D**, **E**) and Sudan black B reactions (**F**). **A**, **B** Some flies fed by standard diet (controls) show fat bodies with loose perivisceral lobes containing cells poor in glycogen and rich in lipid droplets (*arrows*). **C**–**F** Structure and histochemical patterns of fat bodies from flies fed by *E. coli* are similar to those of control flies (see Fig. 2). The oenocytes are indicated by *arrowheads*. *Bars* 50 μm (**A**, **C**, **D**), 10 μm (**B**, **E**, **F**)

the first week of adult life increased longevity by 30–35%, while later lifespan was reduced (Brummel et al. 2004). DeVeale et al. (2004) demonstrated that fly longevity was reduced in the absence of gut bacteria, and it can be restored the first 4 days of adulthood and at mid-life, when the mortality rate increases and tissue specific up-regulation of immune-related genes was found (Pletcher et al. 2002; Seroude et al. 2002; Landis et al. 2004). Similarly,

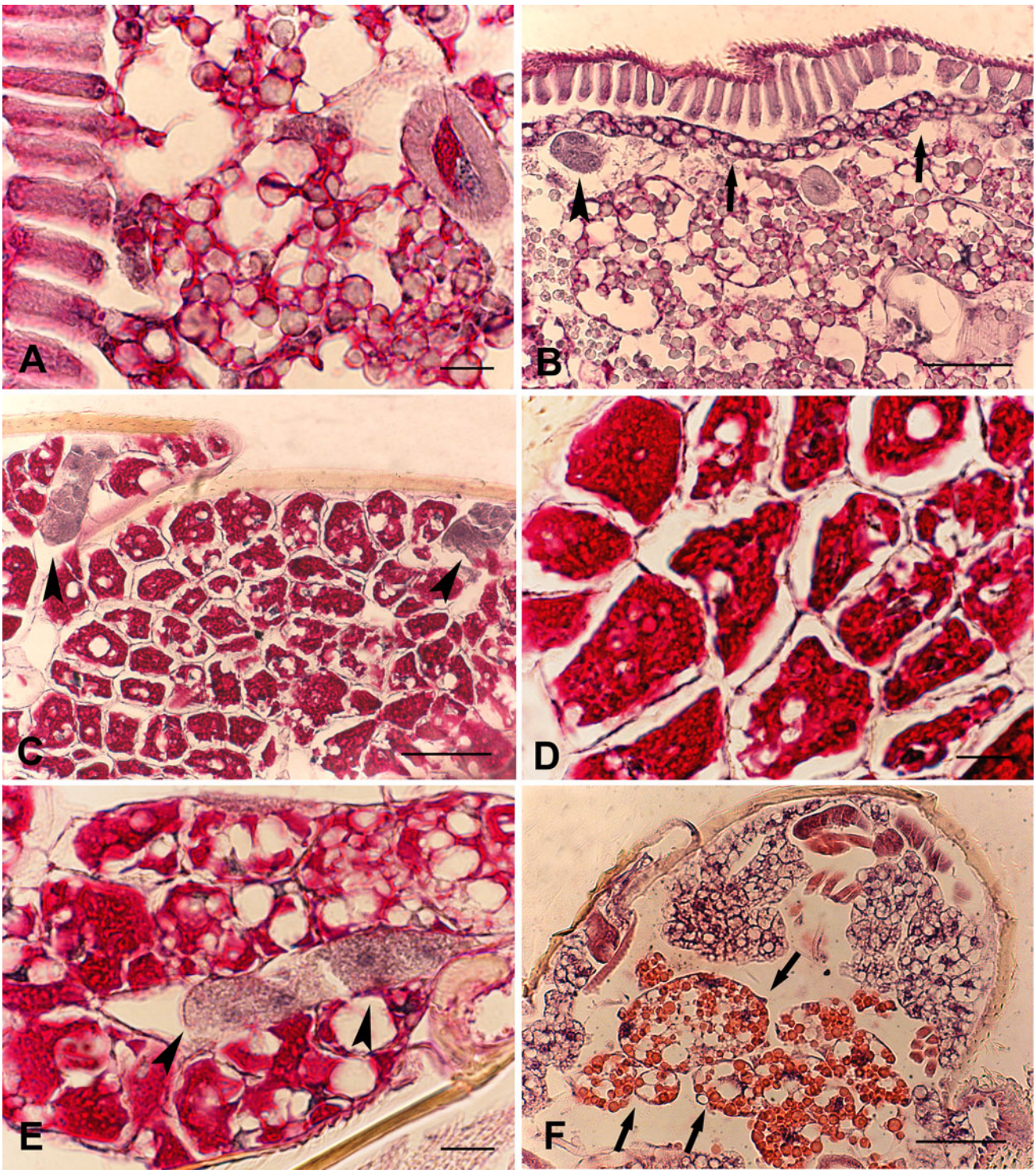

Fig. 4 Histology of the fat body from 9 day old short-life *Drosophila* females Longitudinal sections stained with PAS/hematoxylin (**A**–**E**), hematoxylin-eosin (**F**) stains. **A**, **B** In control survived flies the fat body cells mainly store lipid droplets in its perivisceral lobes, while glycogen storing can be seen in parietal areas (*arrows*). **C**–**E** In contrast, fat bodies from most flies fed by *E. coli* show cells full of glycogen. **F** In some flies fed by bacteria, the cells in perivisceral lobes contain lipid droplets (*arrows*). Segmentally arranged oenocytes (*arrowheads*). *Bars* 50 μm (**B**, **C**, **F**), 10 μm (**A**, **D**, **E**)

the increased longevity here reported were observed when *E. coli* bacteria were added to the diet of flies in their early adult phase. The regulation of host-bacteria interactions is complex and the different responses to early bacteria exposure observed in some long-lived fly mutants suggested the existence of genetic enhancers and suppressors

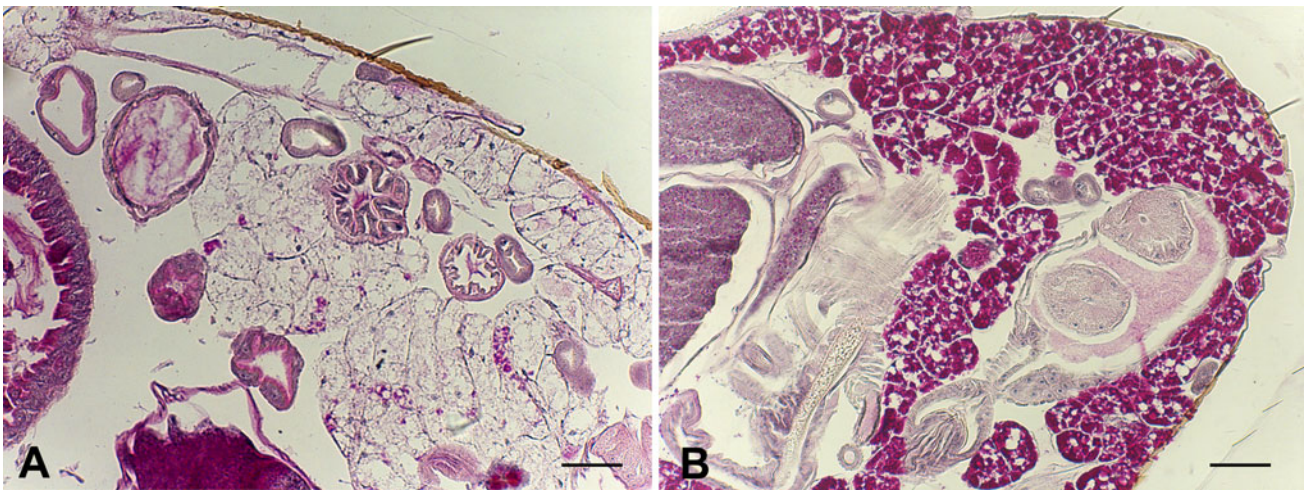

Fig. 5 Longitudinal sections of the fat body from 45 day old long-life *Drosophila* females, stained with PAS/hematoxylin. In control survived flies (**A**), the fat body was reduced in size and no glycogen and lipid droplet stores were detected inside the cells, in contrast to a well developed organ, with strongly PAS positive cells, observed in bacteria fed fly (**B**). *Bars* 50 μm

of microbial effect on animal longevity. Two of these identified modifiers were expressed in the gut and act in the early window of bacterial effect (Brummel et al. 2004).

Beside confirming and extending the lifespan measurement data on the beneficial effect of early addition of *E. coli* to the diet, we here report for the first time that such a dietary regimen has effects on the fat body structure and histochemical reactivity of the stored materials. The main finding is that both short- and long-life bacteria fed flies with extended survival were characterized by great amounts of glycogen accumulated in fat body cells. Differences were found in aged controls fed with standard diet and lipid droplets were seen to be stored in fat body of short-life, but not long-life, flies. In *Drosophila* variations in metabolic patterns have been associated to different longevity phenotypes (Arking et al. 2002) and studies performed on various fly strains indicated that different stress resistant responses and physiological mechanisms may be correlated with extended longevity and with higher lipid or glycogen contents (Service et al. 1985; Service 1987; Graves et al. 1992; Arking et al. 1996). Moreover, flies over-expressing *Drosophila* PGC-1 homolog, transcriptional co-activators involved in mammal longevity, displayed extended life span, and improved tissue homeostasis were observed in old flies with a 20% reduction in triglyceride levels in contrast to a significant increase in glycogen amounts (Rera et al. 2011).

The insect fat body is considered equivalent to mammalian white adipose tissue and liver and is one of the tissue capable of cross-talking with the brain, acting as signalling centre implicated in lifespan regulation (Géminard et al. 2009; Partridge et al. 2010). Fat body is a multifunctional organ target of several hormones and nutritional signals and it controls a variety of functions such as the synthesis, storage and utilization of the energy reserves in the form of triglycerides (constituting more than 90% of the lipid content) and glycogen in response to energetic demands of other tissues. Lipids stored in fat body cells are mobilized and/or utilized for a number of purposes crucial for insect survival, and are required for flight, during starvation, embryonic development and immune response (Canavoso et al. 2001; Liu et al. 2009; Arrese and Soulages 2010). Emerging data in a number of model organisms highlight the contribution of adipose tissue in the aging process, and suggest that decreased triglyceride storage in adipose tissue is related to increased life span (Blüher 2008; Muzumdar et al. 2008; Huffman and Barzilai 2010). In *Drosophila* a reduction in fat tissue mass extended the lifespan by the over-expression of dFOXO, the main transcriptional factor target of IIS, the evolutionarily conserved pathway that affects a variety of processes including lifespan (Paolisso et al. 2001; Bonafè et al. 2003; Klöting and Blüher 2005; Giannakou and Partridge 2007; Blüher 2008). Insulin has been demonstrated to promote lipid accumulation in insect fat body both by increasing the adipocyte number and by promoting triglyceride storage (DiAngelo and Birnbaum 2009). The role of IIS and fat body in the control of fruit fly aging has been also established by the findings that several mutations in single gene encoding IIS components result in an enhanced lifespan (Giannakou and Partridge 2007; Fontana et al. 2010), partial ablation of adult insulin producing cells modulates glucose homeostasis with significant increase in stored glycogen and extends longevity (Haselton et al. 2010) and long lived animals over-express dFOXO in fat body of adult fly (Giannakou et al. 2004; Hwangbo et al. 2004).

On the whole, data indicate that exogenous bacteria extended female survival in both *Drosophila* strains

examined, with a main role of the fat body where accumulation and mobilization of reserve stores are modulated to ensure lifelong energy homeostasis.

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